DESCRIPTION

METHOD FOR MASS PRODUCTION OF SECONDARY METABOLITES IN PLANT CELL CULTURE BY TREATMENT OF AN ALKANOIC ACID OR SALT THEREOF

Technical Field

This invention relates to a method for mass production of secondary metabolites in plant cell culture, and more specifically, to a method for production of secondary metabolites by using plant cell culture, characterized in that a faster growth of plant cells and the enhanced productivity of secondary metabolites are achieved in plant cell culture by treating culture medium with an alkanoic acid or salt thereof.

Background Art

Plants are a useful source for producing a wide variety of secondary metabolites which are used as pharmaceuticals, pesticides, spices, pigments, food additives, cosmetics and the like (table 1). However, while the demands for secondary metabolites in various areas of industries are increasing, the supply of secondary metabolites produced by extraction from plants is limited. Therefore there have been efforts to commercially mass-produce secondary metabolites of plant origin by using plant cell culturing techniques (Stockigt et al., Plant Cell Tissue Org. Cult. 43: 914-920, 1995).

Table 1

Phenylpropanoids	Alkaloids	Terpenopids	Quinones	Steroids
Anthocyanins Coumarins Flavonoids Hydroxycinnamoyl derivatives Isoflavonoids Lignans Phenolenones Proanthocyanidins Stilbenes Tanins	Acridines Betalaines Quinolozidines Furonoquinones Harringtonines Isoquinolines Indoles Purines Pyridines Tropane Alkaloids	Carotenes Monoterpenes Sesquiterpenes Diterpenes Triterpenes	Anthroquinones Bezoquinones Naphthoquinones	Cardiac glycosides Pregnenolone derivatives

However, mass production of secondary metabolites through plant cell culture is still difficult due to problems such as instability of cultured cell lines, low productivity, slow growth, scale-up problems and the like.

Various efforts have been made to try and overcome the low productivity in plant cell cultures, and they include the following methods: 1) adjustment of nutrient sources in the media such as addition of sugar, nitrate salts, phosphate salts, growth control agents and precursors; 2) optimization of the culture environments such as temperature, lighting, pH of the medium, shaking and aeration conditions; 3) treatment with elicitors to enhance productivity; 4) permeabilization of cell membranes and two-phase culture for effective recovery of secondary metabolites; 5) metabolic engineering which enhances productivity of secondary metabolites by modifying genes involved in the biosynthesis of secondary metabolites or introduction of exogenous genes. However, these trials were only effective for particular plant cells or secondary metabolites, and a method that can generally be applied to most plant cell cultures and secondary metabolites has not yet been established.

In nature, plants produce secondary metabolites as a defense mechanism against attack by pathogens, and it has been reported that in some plant cell cultures production of secondary metabolites is enhanced by elicitors originated from pathogens (US. 5,019,504). These elicitors activate genes relating to defense mechanisms while at the same time repressing the expression of genes relating to cell cycle control such as p34cdc2 protein kinase and mitotic cyclin (Logemann et al., Plant Journal 8:865-876, 1995). Also it is known that the HC toxin produced by *Cochliobolus carbonum*, a pathogen of corn, represses the activity of histone deacetylase (Brosch et al., Plant Cell 7:1941-1950, 1995).

On the other hand, butyric acid or sodium butyrate as inhibitors of histone deacetylase induces hyperacetylation of histones H3 and H4 (Riggs et al., Nature 263:462-464, 1977). It was also reported that increased acetylation of histones by butyric acid treatment induces modification of chromosome structure in cells to enhance activity in transcription and has an effect on the change of cell differentiation states (Thorne et al., Eur. J. Biochem. 193:701-713, 1990), and induces apoptosis in some cells (Bhatia et al., Cell Growth Diff. 6: 937-944, 1995). Based on the above range of physiological activity of butyric acid, some researchers have developed a method for enhancing the expression level of exogenous proteins by treating animal cell culture media with butyric acid or sodium butyrate in order to increase transcription of exogenous genes and enhance expression of exogenous protein (US 6,228,618).

Despite the wide range of physiological activity of butyric acid, there has not been much research on the activity and functional mechanism of butyric acid on plant cell cultures, and only study of the effect of butyric acid on growth of plant cells and differentiation of plant tissues or cells have been carried out (Tramontano and Scanlon, Phytochemistry 41:85-88, 1996).

Disclosure

Technical Problem

An objective of the present invention is to provide a method for mass production of secondary metabolites in plant cell cultures.

Another objective of the present invention is to provide optimal conditions in the treatment concentrations, time of treatment, and the number of repeated treatments of alkanoic acid or salt thereof to enhance the productivity of secondary metabolites in plant cell cultures.

Technical Solution

In order to achieve the above objectives, the present invention provides a method for production of secondary metabolites by plant cell culture which includes the step of culturing plant cells upon treatment of the culture medium with alkanoic acid or salt thereof, wherein the alkanoic acid or salt thereof increases the productivity of secondary metabolites by plant cells.

Advantageous Effects

The present invention provides a method for mass production of

secondary metabolites from plant cells by using alkanoic acid or salt thereof which are substances capable of greatly increasing productivity of secondary metabolites in plant cell cultures. By using the present invention, it is possible to greatly increase the productivity of secondary metabolites of plant cells which are known to have very low productivity. Therefore the present invention can greatly benefit the production of commercially useful secondary metabolites of plant origin, such as paclitaxel, corosolic acid and the like.

Description of Drawings

Fig. 1 shows time course of changes in dry cell weight of *Taxus chinensis* cell line SYG-1 after treating with 0 to10 mM of sodium butyrate on day 7 of culture.

Fig. 2 shows the production pattern of paclitaxel in *Taxus chinensis* cell line SYG-1 after treating with 0 to10 mM of sodium butyrate on day 7 of culture.

Fig. 3 is a photograph showing an electrophoresis result of the genomic DNA of *Taxus chinensis* cell line SYG-1 after culture following treatment with 0, 1 and 5 mM of sodium butyrate respectively on day 7 of culture.

Fig. 4 shows time course of changes in the change in the dry cell weight of *Taxus chinensis* cell line SYG-1 after culture following treatment with 1.0 mM of sodium butyrate on different days of culture.

Fig. 5 shows the production pattern of paclitaxel after treating the *Taxus chinensis* cell line SYG-1 with 1.0 mM of sodium butyrate at different

times.

Fig. 6 shows the result of paclitaxel production from *Taxus chinensis* cell line SYG-1 after treating with sodium butyrate at different number of repeated treatments.

Fig. 7 shows taxane produced from *Taxus chinensis* cell line SYG-1 after treating with sodium butyrate.

Fig. 8 shows tome course of changes in dry cell weight of *Taxus chinensis* cell line SYG-1 after treating with sodium propionate.

Fig. 9 shows the result of paclitaxel production from *Taxus chinensis* cell line SYG-1 after treating with sodium propionate.

Fig. 10 shows the result of paclitaxel production from *Taxus chinensis* cell line SYG-1 after culturing in medium containing 1 mM of sodium formate (SF), sodium acetate (SA), sodium propionate (SP), sodium butyrate (SB) or valeric acid (VA) respectively.

Fig. 11 and 12 show HPLC charts of suspension culture solution of *Eriobotrya japonica* and suspension culture solution of *Eriobotrya japonica* where 0.5 mM of sodium butyrate is added.

Mode for Invention

The inventors of the present invention have researched to provide an effective method for enhancing productivity of secondary metabolites which can be applied to various plant cell cultures, and have confirmed that treating the medium for plant cell culture with alkanoic acid or salt thereof can enhance productivity of secondary metabolites in plant cells.

The inventors have accomplished the present invention by discovering that treating plant cell culture media with a relative high concentration of alkanoic acid or salt thereof significantly inhibits cell growth and increases the productivity of secondary metabolites, while an appropriate concentration of alkanoic acid or salt thereof enhances cell growth, and found the optimal conditions for treating with alkanoic acid or salt thereof to produce secondary metabolites in plant cell cultures in mass quantities.

There are a wide variety of secondary metabolites that can be produced from plants, and there a range of different techniques applied according to characteristics of plant species and secondary metabolites produced from them. Table 2 shows examples of the above secondary metabolites and plant cells that can be used in production thereof.

Table 2

Metabolite	Usage	Plant species
Ajmalicine	Antihypertensive	*Cath. roseus*
Artemisinin	Antimalarial	*Artemisia annua*
Ajmaline	-	*Ra. serpentina*
Acinitine	-	*Acotinum* spp.
Berberine	Intestinal ailment	*C. japonica*
Camptothecin	Antitumour	*Camptotheca acuminata*
Capsaicin	Counterirritant	*Ca. frutescens*
Castanospermine	Glycoside inhibitor	*Castanospermum australe*
Codeine	Sedative	*P. somniferum*
Colchicine	Antitumour	*Colchium autumnale*
Digoxin	Heart stimulant	*Di. lanata*
Diosgenin	Steroidal precursor	*Dioscorea deltoidea*
Ellipticine	Antitumour	*Orchrosia elliptica*
Emetine	-	*Cephaclis ipecaccuanha*
Forskolin	Bronchial asthma	*Coleus forskolii*
Ginsenosides	Health tonic	*Panax ginseng*
Morphine	Sedative	*P. somniferum*
Podophyllotoxin	Antitumour	*Podophyllum petalum*
Quinine	Antimalarial	*Cinchon. ledgeriana*
Sanguinarine	Antiplaque	*Sanguinaria canadensis*
Shikonin	Antibacterial	*P. somniferum*
Taxol	Anticancer	*L. erythrorhizon*
Vincristine	Antileukemic	*Taxus* spp.
Vinblastine	Antileukemic	*Cath. roseus*

The method in accordance with the present invention for mass production of secondary metabolites in plant cell culture can be applied to all plant cells that produce secondary metabolites. Preferably, the method can enhance productivity of secondary metabolites by applysing it to various plant cells displaying low productivity of secondary metabolites, and in particular be used in commercial production of paclitaxel by applying to *Taxus* genus cells used to produce paclitaxel which is proven to be effective in treatment of treatment-resistant ovarian cancer and breast cancer.

That is, various species of *Taxus* cells belonging to the *Taxus* genus can have enhanced productivity of secondary metabolites according to the method in the present invention for mass production of secondary metabolites

in plant cell cultures. Examples of the *Taxus* genus includes, but is not limited to, *Taxus bacata*, *Taxus brevifolia*, *Taxus canadensis*, *Taxus chinensis*, *Taxus cuspidata*, *Taxus floridana*, *Taxus globosa*, *Taxus media*, *Taxus wallichiana*, *Taxus yunnanensis*. Mass production of paclitaxel and taxane from plant cells of *Taxus* genus can be possible with the method.

Specifically, a method for mass production of secondary metabolites in plant cell cultures according to the present invention includes the step of treating the plant cell culture with at least one alkanoic acid or salt thereof.

As it is widely known in the related art, the above plant cell culture media can comprise nutrients and other factors necessary to maintain the viability of plant cells, that is, carbon sources, nitrogen sources, salts, vitamins and the like. Also, the plant cell culture media of the present invention can be media widely used in culturing plant cells, for example, but not limited to Murashige & Skoog medium, Gamborg B5 medium, Linsmaier & Skoog medium, which can also be used with various additives on occasion or with some components removed.

For example, in case of producing paclitaxel and taxane compounds by culturing *Taxus chinensis*, B5 medium comprising casein hydrolysate (see: Gamborg et al., Can. J. Biochem., 45 : 417-421(1968)) or a modified Gamborg B5 medium, with 60 g/L of sucrose added to the said B5 medium can be used, and its composition is shown in Table 3 as below. (See: Korean Patent No. 0266448)

Table 3

Components	content (mg/L)
$CaCl_2$ anhydried	113.23
$CoCl_2.6H_2O$	0.025
$CuSO_4.5H_2O$	0.025
$FeSO_4.7H_2O$	27.8
H_2BO_3	3.0
KI	0.75
KNO_3	2500
$MgSO_4.7H_2O$	246
$MnSO_4.H_2O$	10
$NaH_2PO_4.H_2O$	150
$Na_2MoO_4.2H_2O$	0.25
$(NH_4)_2SO_4$	134
$ZnSO_4.7H_2O$	2
Inositol	10
Nicotinic acid	1
Ca-salt of pantothenic acid	0.874
Pyridoxine HCl	1
Riboflavin	0.015
Thiamin.HCl	10
Naphthalene acetic acid	10 uM
Benzyl aminopurine	0.2 uM
Casein hydrolysate	500
$AgNO_3$	1-15 uM
Sucrose	60000

In addition, a plant cell culture medium according to the present invention comprises selectively factors that can induce the production of secondary metabolites. Such factors include plant hormones, biosynthetic precursors or elicitors and signal couplers of secondary metabolites and the like. These factors added in the medium stimulate the production of secondary metabolites in plant cells through various routes and can induce a synergetic effect on the productivity of secondary metabolites together with the treatment with alkanoic acid or salt thereof according to the present invention.

For example, in the case of producing paclitaxel and taxane compounds by culturing *Taxus chinensis*, the preferred inducing agent is silver nitrate and is preferably present in the culturing medium in the concentration

range between 1uM and 15uM and added to the medium in the early stages of culture.

In addition, culture methods and conditions can be selected among known methods based on plant cell types or secondary metabolites.

Alkanoic acid according to the present invention is a compound linked in a single chain having a carbonyl group at one end, and shown as $C_nH_{2n}O_2$. The above n can be an integer from 1 to 9, preferably from 3 to 6. The salt of an alkanoic acid can be an alkaline metal salt, for example, sodium (Na), potassium (K) and the like are suitable. In case of producing paclitaxel and taxane compounds by culturing *Taxus chinensis*, salt of butyric acid or salt of propionic acid, preferably sodium butyrate or sodium propionate can be used.

Alkanoic acids or salts thereof play a role in controlling the plant cell cycle by inducing modification of chromosome structure in cells by increasing the acetylation of histones. Particularly, cessation of cell growth is observed during cell culture of *Taxus chinensis* cell culture is treated with sodium butyrate above a certain concentration (Fig. 1), DNA bands that are multiples of a certain length in size appear on electrophoresis of genomic DNA of cells treated with 5 mM of sodium butyrate, which is characteristic of apoptosis (Fig. 3). The above results indicate that modification of chromosome structure in *Taxus chinensis* cells was induced by sodium butyrate and that this lead to cessation of cell growth and apoptosis.

Alkanoic acid or salt thereof may be present in culture medium at a concentration of between 0.01 to 500 mM, preferably between 0.1 mM to 200

mM, and more preferably between 0.1 mM to 20 mM, but is most preferably to adjust the concentration to suit the particular plant cell type. *Taxus chinensis* may be cultured in a medium containing alkanoic acid or salt thereof at a concentration of between 0.5 mM and 10 mM, and preferably between 0.7 mM and 1.5 mM. Particularly, *Taxus chinensis* cell growth is enhanced on culturing in a medium containing 1 mM of sodium butyrate (Fig. 1), and the productivity of paclitaxel is increased significantly to 1.97 times that of control (Fig. 2).

The treatment time of alkanoic acid or salt thereof to culture medium can be from prior to inoculation of the culture medium with plant cells to the end of growth and activity of the cells, and preferably the early log growth phase of the cells. It is more preferable, however, to adjust the time of treatment with alkanoic acid or salt thereof depending on plant cell type. For example, in the case of *Taxus chinensis* cell culture, the medium may be treated with alkanoic acid or salt thereof from day 0 to day 21 of culture, and more preferably from day 0 to day 14 of culture.

Alkanoic acid or salt thereof induces physiological changes of plant cells, and particularly induces changes such as inhibition of histone deacetylase activity or suppression of cell cycle related gene expression. The above changes are reversible; therefore the changed characteristics of the cells return to its original state when the alkanoic acid or salt thereof is depleted. So, it is preferable that the alkanoic acid or salt thereof is present in the medium at a constant concentration. Therefore, after the initial treatment of the culture medium with alkanoic acid or salt thereof, the culture medium

may be treated one or more times at regular intervals. And alkanoic acid or salt thereof may be maintained in the medium at a concentration of between 0.01 and 500 mM, preferably between 0.1 mM and 200 mM, and more preferably between 0.2 mM and 20 mM. It is most preferable, however, that the concentration of the alkanoic acid or salt thereof be varied having regard to the particular plant cells. *Taxus chinensis* cells can be treated with alkanoic acid or salt thereof in the medium one to three times during culture, and preferably two to three times.

All appropriate plant cell culture methods known to the relevant field of technology may be used in the cell culture of the present invention. For example, batch culture, continuous culture, fed-batch culture, semi-continuous batch process, immobilized culture, two-phase culture and the like may be used and selected depending on plant cell type and characteristics of the secondary metabolites.

In one embodiment of the present invention, paclitaxel and taxane compounds were produced by culturing *Taxus chinensis* cells according to the present invention. As a result, the productivity of paclitaxel was increased six-fold and the productivity of taxane compounds was increased from about 1.6-fold to about 9-fold depending on the taxane compound.

A method of the present invention can be applied to increase the productivity of corosolic acid, which can be used to treat anti-insulin-independent (Type II) diabetes, by *Eriobotrya japonica* cell culture.

Also, the present invention provides plant cell culture media and

media for the production of secondary metabolites from plant cell culture containing alkanoic acid or salt thereof. The alkanoic acid or salt thereof may be present in the medium at a concentration of between 0.01 mM and 500 mM, preferably between 0.1 mM and 200 mM, more preferably between 0.1 mM and 20 mM. It is most preferable, however, that the concentration of the alkanoic acid or salt thereof may be appropriately varied having regard to the particular plant cells.

The present invention is further illustrated in the following examples, which should not be taken to limit the scope of the invention.

EXAMPLE 1: The effect of treating plant cell cultures with sodium butyrate

1-1. Determination of plant cell growth and productivity of secondary metabolites

Taxus chinensis SYG-1 cell line (KCTC-0232BP) was used to produce paclitaxel and taxane compounds.

50 ㎖ of SYG-1 culture solution incubated in a medium containing 30 g/L of sucrose (Korean patent NO.266448) for 14 days were inoculated to 50 ㎖ of medium containing 60 g/L of sucrose in 250 ㎖ Erlenmeyer flasks and incubated in the dark at 24℃, 150 rpm for 14 days. Then, the cultures were incubated at an elevated temperature of 29℃ for 28 days. Also, sodium butyrate was added at concentrations of 0, 0.5, 1, 5 and 10 mM, respectively on day 7 of culture in order to increase paclitaxel productivity of SYG-1, and

each culture was sampled on days 14, 21, 28, 35, 42 of culture to measure cell growth and paclitaxel productivity.

Cell growth was observed by determining dry cell weight (DCW). Dry cell weight is weight of cells determined after drying in a drying oven at 60 ℃ for 24 hours following filtration of the sample from plant cell culture through Whatman No. 4 filter paper using a Buchner funnel.

Paclitaxel productivity was measured by a quantitative analysis method for paclitaxel and taxane compounds widely known to the relevant field of technology (Korean Patent No. 0266448).

Cell growth and paclitaxel productivity of SYG-1 culture treated with sodium butyrate was shown in Fig. 1 and Fig. 2 (Fig. 1 and Fig. 2 - □: not treated, ○: 0.5 mM sodium butyrate, △: 1.0 mM sodium butyrate, ◇: 5.0 mM sodium butyrate, X: 10.0 mM sodium butyrate).

When treated with 0.5 mM of sodium butyrate, there are no significant changes in cell growth and paclitaxel productivity. However, when treated with 1 mM of sodium butyrate, cell growth was greatly increased from the 14^{th} day of culture, and reached a peak of 23.22 g/L on day 28 and then gradually decreased. When treated with 5 mM and 10 mM of sodium butyrate, cell growth did not occur and the color of cells turned dark brown as apoptosis occurred. And on treating with 1mM of sodium butyrate, the quantity of paclitaxel produced was at a maximum of 133.8 mg/L on day 35 of culture, which is 1.97 times that of the control.

1-2. Determination of intracellular changes

Genomic DNA of 1g (fresh weight) of *Taxus chinensis* SYG-1 cell cultured in medium treated with 0 mM, 1 mM and 5 mM of sodium butyrate was extracted by using a DNA extracting kit (DNeasy Plant Mini Kit, QIAGEN, U.S.A.). 250 ug of the extracted genomic DNA was separated by electrophoresis on an agarose gel (Fig. 3). In Fig. 3, lane A is a size marker, lane B is a non-treated sample, lane C is a sample treated with 1 mM of sodium butyrate and lane D is a sample treated with 5 mM of sodium butyrate.

Under the condition of treatment with 5 mM of sodium butyrate, cessation of cell growth was confirmed (Fig. 1) and DNA bands that are multiples of a certain length in size could be seen in the electrophoresis of genomic DNA, which is characteristic of apoptosis. So, we could see that sodium butyrate in the medium induced modification of chromosome structure in *Taxus chinensis* cells which caused cessation of cell growth and apoptosis.

EXAMPLE 2: Determination of time of sodium butyrate treatment

Sodium butyrate was added at concentrations of 1 mM on day 0, day 7, day 14 and day 21 of culture respectively in *Taxus chinensis* SYG-1 was cultured and a sample of each was removed from the culture on days 14, 21, 28, 35, 42 respectively, and cell growth and productivity of paclitaxel was determined in the same method as in EXAMPLE 1.

Fig. 4 shows the cell growth according to the treatment time of sodium butyrate and Fig. 5 shows paclitaxel production (in the Fig. 4 and Fig. 5 - □: non-treated sample, ○: sample treated with 1 mM of sodium butyrate on day 0 of culture, △: sample treated with 1 mM of sodium butyrate on day 7 of culture,

◇: sample treated with 1 mM of sodium butyrate on day 14 of culture, X: sample treated with 1 mM of sodium butyrate on day 21 of culture).

In case of *Taxus chinensis* SYG-1 treated with 1 mM of sodium butyrate, high cell growth was observed regardless of time of treatment with sodium butyrate in comparison with the non-treated control. Furthermore, paclitaxel productivity was highest when 1 mM of sodium butyrate was treated on day 7 of culture.

On the basis of results in Fig. 4, the maximum specific growth rate of cell growth of *Taxus chinensis* SYG-1 depending on the time of treatment with 1 mM of sodium butyrate, was obtained and shown in Table 4. The earlier the time of treatment with sodium butyrate, the higher the maximum specific growth rate of cells, and maximum dry cell weight was attained sooner. However, maximum dry cell weight was highest being 23.22 mg/L, when 1 mM of sodium butyrate was treated on day 7 of culture.

Table 4

Treatment condition	Maximum specific growth rate (u_{max}) (day^{-1})
Not treated(control)	0.035
day 0 of culture	0.078
day 7 of culture	0.066
day 14 of culture	0.038
day 21 of culture	0.039

EXAMPLE 3: Repetitive treatment of sodium butyrate

While culturing *Taxus chinensis* SYG-1 cell by using the same method as the EXAMPLE 1-1, 1 mM of sodium butyrate was added on day 1 and day 15 of culture, and then same concentration of sodium butyrate was added up to three more times at 7-day intervals. Productivity of paclitaxel on

day 30 and day 37 of culture were determined (Fig. 6 - □: day 30 of culture, ▨: day 37 of culture)

As a result, productivity of paclitaxel was increased in proportion to the number of treatments. When 1 mM of sodium butyrate was repeatedly treated three more times, productivity of paclitaxel was 6.43 times higher compared with control on day 37 of culture. And in case of treating with 1 mM of sodium butyrate on day 15 of culture followed by three repeated treatments, the productivity of paclitaxel, was 4.95 times higher compared with the highest productivity attained with only two repeated treatments, as control.

EXAMPLE 4: Production of taxane compounds by treatment of sodium butyrate

While culturing *Taxus chinensis*. SYG-1 cell by using the same method as in EXAMPLE 1-1, 1 Mm of sodium butyrate was added on day 7 and day 14 of culture. The culture was sampled on day 28, day 36 and day 43 of culture in order to determine the productivity of taxane compounds.

Fig. 7 shows the productivity of taxane compounds (rate of increase or decrease of taxane compounds compared with control calculated as %); □–cultured for 28 days, ▨–cultured for 36 days, ■–cultured for 43 days, A is BacIII, B is 10-DAT, C is taxcultine, D is cephalomammine, E is 13-deacetyl taxchinin I, F is paclitaxel, G is benzyl analog, and H is H4.

In Fig. 7, the productivity of all the taxane compounds was increased significantly upon treatment with sodium butyrate, and particularly when

cultured for 28 days it increased greatly from about 1.6 to 9 times. However, the degree of increase became smaller, being 1 to 3 times on day 36 of culture, and became even smaller on day 43. This indicates that the effect of sodium butyrate on secondary metabolite productivity increase is a kind of induction effect, and is not continuous. That is, sodium butyrate induces production of secondary metabolites for a period of time after treatment, but there is a tendency to return to the original state. Furthermore, it can be seen that the phenomenon of productivity increase is not limited to paclitaxel, but generally applies to all secondary metabolites.

EXAMPLE 5: Treatment of sodium propionate

While culturing *Taxus chinensis* SYG-1 cells, 0.5 mM or 1 Mm of sodium propionate was treated on day 7 and day 14 of culture.

Fig. 8 shows the cell growth of SYG-1 cultured with sodium propionate treatment and Fig. 9 shows the productivity of paclitaxel (in the Fig. 8 and Fig. 9 - □: non-treated sample, ○: sample treated with 0.5 mM of sodium propionate on day 7 of culture, △: sample treated with 0.5 mM of sodium propionate on day 7 and day 14 of culture, ●: sample treated with 1 mM of sodium propionate on day 7 of culture, ▲: sample treated with 1 mM of sodium propionate on day 7 and day 14 of culture).

When treated with sodium propionate, cell growth of *Taxus chinensis* SYG-1 cell was increased, and cell growth was rapid when the concentration of sodium propionate was 1 mM and when treated two times repeatedly.

The higher the concentration of sodium propionate was and the

higher the number of treatments, the higher the productivity of paclitaxel was.

From the above results, it can be seen that alkanoic acid or salt thereof other than sodium propionate can also increase the productivity of secondary metabolites in plant cell culture.

EXAMPLE 6: Effect of various alkanoic acids

In culturing *Taxus chinensis* SYG-1 cells, 1 mM of sodium formate (C1), sodium acetate (C2), sodium propionate (C3), sodium butyrate (C4) and valeric acid (C5) was added respectively on day 7 of culture.

Fig. 10 shows paclitaxel productivity of *Taxus chinensis* SYG-1 cells cultured in the medium including 1 mM of sodium formate (SF), sodium acetate (SA), sodium propionate (SP), sodium butyrate (SB) or valeric acid (VA) respectively. Salts of alkanoic acids containing one to five carbon atoms augmented paclitaxel productivity.

EXAMPLE 7: Production of corosolic acid

Callus and suspension culture was induced from *Eriobotrya japonica*, a plant indigenous to Wando, Jeollanam-do province.

Collected leaves were treated with 70% alcohol for 60 seconds, with 1% sodium hypochlorite for 20 minutes, and then surface sterilized by washing three times with sterilized distilled water. Thereafter, the leaves were incubated in the dark at 24℃ in the culture medium of EXAMPLE 1-1 containing 0.7% agar. After 3~4 weeks of culture, induced callus was transferred to agar-free liquid culture medium and suspension cultured at 24℃, 120 rpm in the dark. The established suspension cultured cells of *Eriobotrya japonica* was

subcultured in the above medium at two-week intervals.

The suspension culture cell of *Eriobotrya japonica* was treated with 0.5 mM of sodium butyrate followed by 14 days of culture, then corosolic acid production was confirmed. The method of extracting corosolic acid was carried out according to the quantitative analysis method for paclitaxel and taxane compounds in EXAMPLE 1-1, and detection of corosolic acid was carried out by determining absorbance at 210 mM wavelength in flow of 40~80% acetonitrile solution at 1 ml/min flow rate.

Fig. 11 and 12 show HPLC charts of extracts from *Eriobotrya japonica* cells cultures in the absence(Fig. 11) and the presence(Fig. 12) of 0.5 mM of sodium butyrate, respectively. A corosolic acid peak was detected at 20.1 minutes of retention time. The productivity of corosolic acid was increased 63% on treatment with 0.5 mM of sodium butyrate.